ORIGINAL ARTICLE

The essential oil from *Vanillosmopsis arborea* Baker (Asteraceae) presents antinociceptive, anti-inflammatory, and sedative effects

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Background: The essential oil from *Vanillosmopsis arborea* (EOVA) Baker is rich in (-)-α-bisabolol. Investigations demonstrated its gastroprotective, larvicidal, and visceral antinociceptive activities. **Aims:** The present study aimed to elucidate the antinociceptive, anti-inflammatory and sedative properties of the EOVA Baker in mice. **Materials and Methods:** The antinociceptive and anti-inflammatory activities were assessed using the abdominal constriction, formalin and carrageenan-induced paw edema models, respectively. The sedative property was detected by the open-field and sleeping time tests. Results were analyzed by ANOVA, followed by Student-Newman–Keuls test. **Results:** EOVA, after intraperitoneal administration, produced an inhibition of the acetic acid-induced writhing in mice. In addition, the same doses were able to inhibit both the early and late phases of the formalin-induced nociception. EOVA produced inhibition in the carrageenan-induced edema model, reduced the spontaneous motor activity and prolonged the sleeping time induced by pentobarbital. **Conclusion:** The experimental data demonstrated that EOVA showed antinociceptive, anti-inflammatory, and sedative activities.

Key words: Anti-inflammatory agents, antinociceptive agents, essential oils, sedative effect

INTRODUCTION

Vanillosmopsis arborea Baker belongs to the Asteraceae family and is popularly known as "candeeiro." It is a small tree which grows in the Araripe National Forest, in the state of Ceará, Brazil. Its wood has a strong odor of chamomile and burns easily with a strong flame.[1]

Chemical studies of the essential oil revealed the presence of a high content of (-)-α-bisabolol[2] and experimental investigations demonstrated its gastroprotective,[3] larvicidal,[4] and visceral antinociceptive[5] activities. Besides, this plant is popularly used as a repellent.

Based on its pharmacological activities demonstrated in previous reports, this study aims to evaluate the essential oil from *V. arborea* (EOVA) in animal models of acute pain, inflammation, and central nervous system activity in order to contribute toward the pharmacological knowledge about this plant.

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MATERIALS AND METHODS

Plant material

Vanillosmopsis arborea Baker (Asteraceae) was collected at the Chapada do Araripe, Ceará, Brazil, and identified by a Taxonomist of the Federal University of Ceará, Brazil. EOVA was extracted from freshly chopped plant barks by steam distillation and analyzed at the Natural Products Research Laboratory of Regional University of Cariri. The chopped barks were placed in a glass flask connected at one end to a glass vessel with water and at the other end to a water-cooled condenser. When the water was boiled, steam percolated through the barks and was collected in the condenser. After condensation, the essential oil was separated from the aqueous phase with its solutes.

Gas chromatography/mass spectrometry (GC/MS) analysis

Analysis by gas chromatography/mass spectrometry (CG/MS) of the essential oils was carried out on a Shimadzu GC-17 A/MS QP5050A (GC/MS system) using a DB-5HT fused silica capillary column (30 m × 0.25 mm i.d., 0.25 m film thickness); carrier gas helium, flow rate 1.7 mL/min and with split mode. The injector temperature and detector temperature were 270°C and 290°C, respectively. The column temperature was programmed from 35°C to

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180°C at 4°C/min and then 180°C to 250°C at 10°C/min. Mass spectra were recorded from 30 to 450 m/z. injected volume: 1 mL of 5 mg/mL solution ethyl acetate. Solvent cut time was 3 min. Individual components were identified by matching their 70 eV mass spectra with those of the spectrometer database using the Wiley L-built library and two other computer libraries MS searches using retention indices as a preselection routine, as well as by visual comparison of the fragmentation pattern with those reported in the literature. The composition (w/w) of EOVA revealed the presence of (-)-α-bisabolol to extent of 70% [Figure 1]. Others identified compounds were α-cadinol (8.4%), elemicin (6.21%), β-bisabolene (4.46%), δ-guaiene (2.31%), β-cubebene (1.76%), and estragole (1.08%).

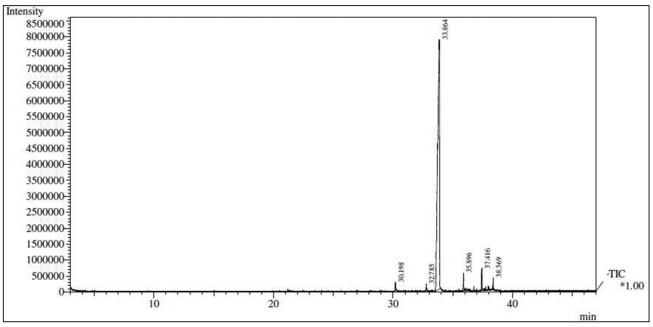

Figure 1: Gas chromatography/mass spectrometry - chromatogram total ion currency (TIC) profile of *Vanillosmopsis arborea* essential oil

Animals

Male Swiss albino mice (20–25 g) obtained from the Central Animal House of Faculty of Medicine of Juazeiro do Norte were used. They were housed in environmentally controlled conditions (22°C, 12 h light–dark cycle), with free access to standard pellet diet (Purina, São Paulo, Brazil) and water. Animals were kept in cages with raised floors to prevent coprophagy. They were fasted over a period of 15 h and were habituated to the test environment for 2 h before the experimentation. The experimental protocols were approved by the Animal Care and Use Committee of the Faculty of Medicine of Juazeiro do Norte (Protocol n° 2009_0187_FR246195) in accordance with the ethical guidelines of National Institute of Health, Bethesda, USA.

Writhing Test

Mice were treated with EOVA (5, 10 25 and 50 mg/kg, intraperitoneal [i.p.], 30 min (*p.o.*), before receiving a 1% acetic acid injection (10 mL/kg, i.p.). The number of writhing determined by abdominal muscle contractions and hind paw extension was recorded for 20 min, starting 10 min after the administration of acetic acid. Indomethacin (10 mg/kg, i.p.) was used as standard drug.

Formalin Test

This test, which causes a local tissue injury to the paw, has been used as a model for tonic pain and localized inflammatory pain. For this, 20 µL of a 1%-formalin solution was injected into the right hind paw of mice, and the licking time was recorded after the first 5 min (1st phase, corresponding to a direct chemical stimulation of nociceptors) and after 20 min (2nd phase, involving inflammation), for 5 min each time. Animals were pretreated with EOVA (5, 25, and 50 mg/kg) 30 min (i.p.) before intraplantar formalin injection. Indomethacin (10 mg/kg, i.p.) was used as standard drug.

Paw Edema Induced by Carrageenan

The animals were pretreated with EOVA (5, 25, 50, and 100 mg/kg, i.p.) or indomethacin (10 mg/kg, *p.o.*) before receiving the injection of 40 µl of 1% carrageenan (w/v) into the subplantar area of the right hind paw. The paw volume was determined with a plethysmometer (1–24 h).

Open Field Test

The open field area was made of acrylic (transparent walls and black floor, 30 cm × 30 cm × 15 cm) divided into nine squares of equal area. The open field was used to evaluate the exploratory activity of the mouse. Mice pretreated with EOVA (25, 50, and 100 mg/kg; i.p.) or diazepam (1 mg/kg, i.p.) were placed in the center of the arena and the number of squares crossed, with the four paws was recorded for 5 min. The observed parameters were: Number of squares crossed (locomotor activity) and number of grooming (stereotype) and rearing (exploratory behavior).

Barbiturate-Induced Sleeping Time

Mice were treated with EOVA (25, 50, and 100 mg/kg; i.p.) or diazepam (1 mg/kg, i.p.), and 30 min later all groups received sodium pentobarbital (40 mg/kg, i.p.). The time since the injection up to the loss of the righting reflex was recorded as sleeping latency and the time elapsed between the loss and voluntary recovery of the righting reflex was recorded as sleeping time.

Statistical Analysis

The results are presented as mean ± standard error of mean and were analyzed by ANOVA, followed by Student-Newman–Keuls as the *post-hoc* test (GraphPad Prism program, USA). $P < 0.05$ was used as the significance level.

RESULTS

Essential oil from *V. arborea* (25 and 50 mg/kg) inhibited the acetic acid-induced abdominal constrictions [Figure 2]. In the formalin test [Figure 3], although both phases of the response were significantly inhibited, the EOVA effect was predominant in phase 2. EOVA was very effective in causing inhibition of the paw volume in the carrageenan-induce paw edema in mice [Table 1].

The oil in higher doses showed significant inhibition of the paw edema at the 2nd, 3rd, and 4th h after carrageenan administration. It showed a significant effect even at the smallest dose (5 mg/kg), which inhibited paw edema at the 2nd h after carrageenan administration. Higher inhibitions were detected with the doses of 50 and 100 mg/kg (48% and 51%, respectively) at the same period of time. Indomethacin promoted similar effects.

The locomotor activity, rearing and grooming [Table 2] were investigated for the behavioral studies. EOVA (50 and 100 mg/kg) decreased not only the locomotor activity (36 and 45%, respectively), but also the number of rearing (38 and 58%, respectively) as compared with control. Similarly, animals treated with diazepam 1 mg/kg, i.p. decreased these parameters too.

The absolute values of the sleeping latency and duration of sleep are presented in Table 3. The treatment with EOVA (50 and 100 mg/kg) before injection of pentobarbital resulted in a statistically significant prolongation of the pentobarbital-induced sleeping time by 33% and 93%, respectively. No significant effect was observed in the presence of the smallest dose. A similar effect was observed in the group treated with diazepam (1 mg/kg, i.p).

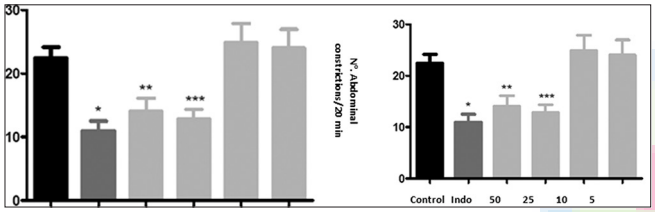

Figure 2: Effect of essential oil from *Vanillosmopsis arborea* in the writhing test. ANOVA, followed by Student-Newman–Keuls. Data are expressed as media ± standard error of mean (*P < 0.05; **P < 0.01, and ***P < 0.001 versus control)

DISCUSSION

In this work, the effects of the EOVA were studied in several animal models, as the writhing and formalin tests, paw edema induced by carrageenan, the open field test and the barbiturate-induced sleeping time in mice. The tests cited above are classical animal models that provide information about antinociceptive, anti-inflammatory, psychomotor performance, and depressant activities.

Essential oil from *V. arborea* presents an important antinociceptive effect as demonstrated by the writhing and formalin tests in mice. In the formalin test, the effect was manifested in both phases, although more intensely in the 2nd phase of the response. The formalin test is considered a valid model for clinical pain.[6] The first phase of the behavioral response is thought to be produced by direct activation of nociceptive neurons, while the second phase corresponds to inflammatory pain and thus involves the release of mediators of the inflammatory process.[7,8] Our results suggest that the antinociceptive effect of EOVA seems to be related mainly to their anti-inflammatory action.

Confirming this hypothesis, EOVA showed an antiedematogenic activity in paw edema induced by carrageenan in mice. As a matter of fact, EOVA at the two

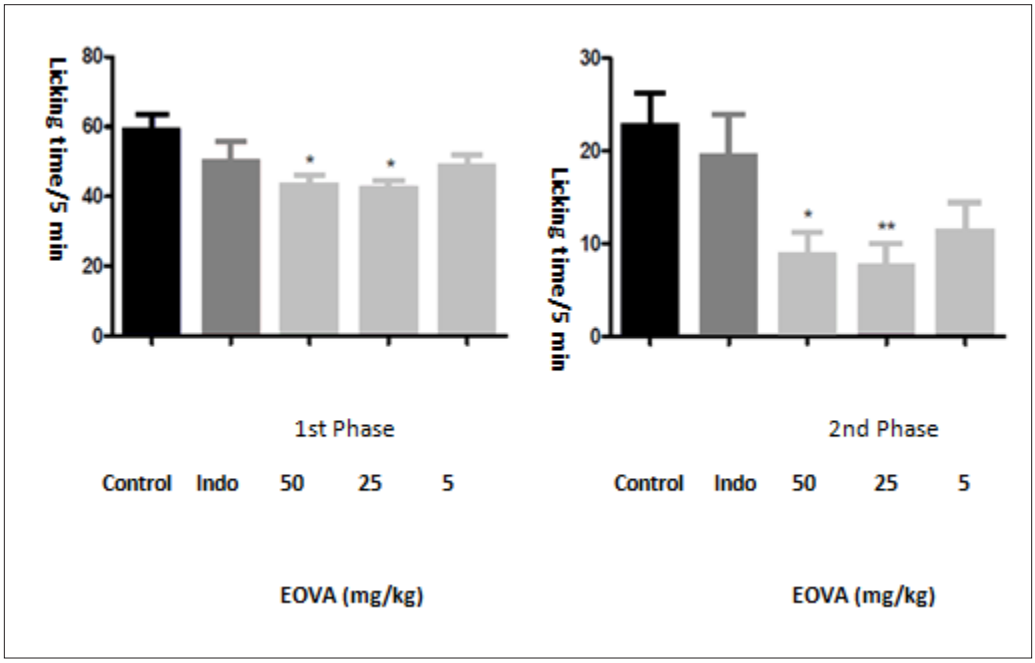

Figure 3: Effect of essential oil from *Vanillosmopsis arborea* in the formalin test. ANOVA, followed by Student-Newman–Keuls. Data are expressed as media ± standard error of mean (*P < 0.05 and **P < 0.01 versus control)

Table 1: Effect of EOVA in the rat paw edema induced by carrageenin

Treatment	Dose (mg/kg)	T_1	T_2	T_3	T_4	T_{24}
Control		0.0130±0.0011	0.0210±0.0011	0.0270±0.0018	0.0290±0.0020	0.0180±0.0026
Indomethacin	10	0.0070±0.0012	0.009±0.0015***	0.0120±0.0014***	0.0170±0.0033**	0.0080±0.0012
EOVA	100	0.0100±0.0012	0.0102±0.0013**	0.0140±0.0010***	0.0100±0.0018***	0.0070±0.0013
	50	0.0060±0.0012**	0.0110±0.0017***	0.0180±0.0015***	0.0200±0.0014**	0.0110±0.0022
	25	0.0100±0.0014	0.0150±0.0013**	0.0210±0.0014*	0.0240±0.0017	0.0130±0.0027
	5	0.0100±0.0020	0.0160±0.0018**	0.0230±0.0021	0.0260±0.0022	0.0150±0.0029

ANOVA, followed by Student-Newman-Keuls. Data are expressed as media±s.e.m. *P<0.05; **P<0.01 and ***P<0.001 *vs* control, EOVA – Essential oil from *Vanillosmopsis arborea*

Table 2: Effect of EOVA on the frequency of locomotion, numbers of rearing and grooming

Treatment	Dose (mg/kg)	Number of behaviors/5 min		
		Crossing	Rearing	Grooming
Control	-	69.00±2.08	24.00±1.00	3.00±0.20
Diazepam	1	56.00±2.70	11.00±2.20***	2.00±0.50
EOVA	100	38.00±6.50***	10.00±1.80***	2.00±0.30
	50	44.00±2.05***	15.00±1.40***	2.00±0.20
	25	59.00±1.40	16.00±1.10***	1.00±0.20*

ANOVA, followed by Student-Newman-Keuls. Data are expressed as media±s.e.m. *P<0.05 and ***P<0.001 *vs* control, EOVA – Essential oil from *Vanillosmopsis arborea*

Table 3: Effect of EOVA on pentobarbital-induced hypnosis in mice

Treatment	Dose (mg/Kg)	Sleep latency (s)	Sleeping time (s)
Control		195.0±15.0	1857.0±771.0
Diazepam	1	112.0±14.0*	7820.0±1288.0***
EOVA	100	188.0±20.3	3587.0±915.0**
	50	208.0±30.0	2477.0±453.0*
	25	166.0±10.6	1931.0±470.0*

ANOVA, followed by Student-Newman-Keuls. Data are expressed as media±s.e.m. *P<0.05; **P<0.01 and ***P<0.001 *vs* control, EOVA – Essential oil from *Vanillosmopsis arborea*

higher doses significantly decreased paw volume at all periods of time after the administration of carrageenan. The edema induced by carrageenan presents an acute phase lasting up to 24 h and a 2nd phase which starts after 24 h and lasts at least 72 h after its administration.[9] The edema involves the release of several mediators such as 5-HT, histamine, bradikynin, and prostaglandins[10] and active compounds may act on one or several steps of the inflammatory process.

Essential oil from *V. arborea* significantly decreased locomotor activity as demonstrated by the open field test in mice. Our findings showed that EOVA (50 and 100 mg/kg) decreased not only the locomotor activity, but also the number of rearing. A decrease in these parameters indicates central nervous system depression induced by *V. arborea*. It is widely accepted in the literature that rearing is a function of the excitability levels of the central nervous system.[11]

Data in the literature point out that benzodiazepines, like diazepam, act as anxiolytics (at low doses) and anticonvulsants, producing also a myorelaxant effect at higher doses.[12,13] Based on this, we aimed to evaluate the effects of EOVA in potentiating pentobarbital sleeping time. It is known that the decrease in sleep latency and increase in sleeping time is classically related to central nervous system depressant drugs.[14] Our results showed that EOVA, in all doses, increased the duration of sleep, suggesting a potentiation of pentobarbital-induced sleeping time. However, this test is not specific because compounds that interfere with the biotransformation of pentobarbital by cytochrome P-450 complex can show the same effects on central nervous system as that of depressant drugs.[15]

Perez *et al*.[16] showed that a decrease in spontaneous motor activity resulted from a reduced excitability of the central nervous system and sedation.[17] The decrease in spontaneous motor activity and potentiation of pentobarbital-induced sleep strongly indicates central depressant activity of EOVA, thus suggesting that the essential oil might be acting as a mild neurosedative drug.[18]

CONCLUSION

We showed an analgesic and anti-inflammatory activities of the essential from *V. arborea*. These effects may be related to the high (-)-α-bisabolol content in EOVA, since Leite *et al*.[19] and Rocha *et al*.[20] have reported that (-)-α-bisabolol presents anti-inflammatory and antinociceptive properties. Besides this, we showed that acute treatment with EOVA potentiated the barbiturate-induced sleeping time and presents a depressant effect.

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